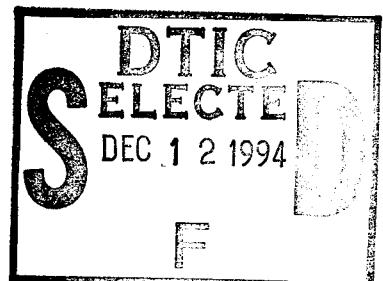




Effect of Vibration Frequency and Amplitude on Developing Chicken Embryos

By

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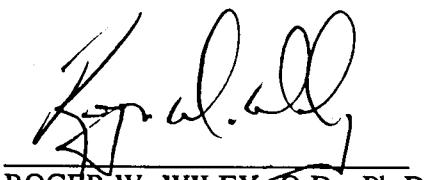
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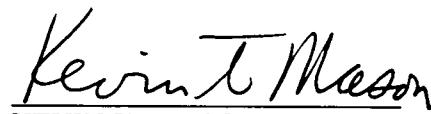
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| Factors associated with chicken embryo mortality were: frequency, amplitude, amplitude transmission, and timing of the exposure. As the magnitude of the exposure increased, mortality increased. No clear HRA threshold values were identified due to the retrospective discovery of protocol problems. The use of an entire tray of eggs as the unit of amplitude measure was invalid due to the differences in vibration amplitude transmission at each egg station. However, mortality thresholds were proposed using a logistic model that controlled for the differences in vibration amplitude noted at various egg stations. No extraneous factors such as month of incubation, flock, and incubator were significant in analysis of variance modeling. | | | | | |
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Controlling for the amplitude transmission, we found very strong effects of vibration on embryonic development and mortality. Exposure of chicken embryos to vibration above 2.0 G_Z should be avoided. Exposures as low as 1.0 G_Z are harmful at certain frequencies.

Congenital malformations occurred in chicks exposed to vibration, but in none of the control chicks. The malformation syndrome was characterized by crossed beaks and missing eyes. We observed several experimental chicks with malformed feet, sensory disorientation, and muscular weakness. Further studies are required to define and validate these findings.

Whole-body vibration exposures are harmful to the developing chicken embryo. Until further laboratory and epidemiological studies of this potential health hazard on animal and human pregnancy outcomes are completed, pregnant Army aviators should not fly in rotary-wing aircraft. We believe the vibratory aspect of the rotary-wing aircraft environment is a fetal health hazard.

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Introduction

Military flight surgeons from multiple clinical specialty backgrounds, female aviators, and spouses of female aviators are concerned about the effects of various aviation physical factors and toxins on pregnant aviators and the unborn child. The potential risks of flying duty on pregnancy are defined, but the association between exposure and adverse effects is not well documented in the scientific literature (Mason, 1994). Whole-body vibration (WBV) is a significant physical force found in rotary-wing flight. In this report, we explore equipment and methods required to assess the association between WBV and abnormal chicken embryo development as a model for human development in vibration environments.

WBV damages animal embryos, causing developmental failures and birth defects. Whether the suspension systems of the human uterus and amniotic fluid attenuate or accentuate WBV is unknown. The effects of WBV on the development of the human fetus are unknown. Some studies have shown women with occupational exposure to WBV, such as heavy equipment operators and truck drivers, are at increased risk for miscarriage and birth defects (Council of Scientific Affairs, 1984; Flournoy, 1990; Pelmear, 1990; Von Briese, Fanganel, and Gasow, 1984; McDonald et al., 1988; World Health Organization, 1982; and Bantle, 1971).

Currently, U.S. military aviators are restricted to certain flying duties during pregnancy. They are restricted from flying in high performance and rotary-wing aircraft during all phases of pregnancy beginning with the date of diagnosis until recovery is complete after delivery. Aviators may fly multiengined, low performance, dual-pilot, fixed-wing aircraft with cabin altitudes not exceeding 10,000 feet above mean sea level during the second trimester of pregnancy, if the pregnancy is uncomplicated. They are restricted from these aircraft during the first and third trimesters. They may fly in synthetic flight trainers during pregnancy (Department of the Army, 1993; and Mason, 1994).

Literature review

WBV exposure standards

Standards for health risks associated with WBV are stated in ISO 2631, an international standard (International Organization for Standardization, 1985). These limits are based on a series of health risk assessments (HRA), and address three levels of exposure: health and safety, comfort, and fatigue-decreased proficiency. Just how accurately ISO 2631 predicts the risk of WBV in pregnancy is questionable. ISO 2631 has been criticized because: 1) the standard lacks empirical support in many areas, 2) the use of the same shape of frequency weights for the three criteria (levels) is an oversimplification of the true situation, and 3) the dependency exposure duration or timing of exposure during pregnancy on adverse pregnancy outcomes is unknown. These problems require further study as a foundation for improved standards.

U.S. Army studies

About 2.5 percent of U.S. Army aviators are women. Most are of childbearing age since only 1.0 percent of U.S. Army female aviators are over age 40 (Mason and Shannon, 1994). Among female aviators, pregnancy is a major cause for restriction from flying duties (Mason, 1990; Mason, 1994; and Shannon and Mason, 1994).

The outcomes of pregnancy, and their association with attrition from aviation service, are under study by reviewing medical data in the Aviation Epidemiology Data Register (AEDR). Preliminary findings reveal that the outcomes of pregnancy are documented irregularly in the AEDR, especially pregnancies that end in the first and second trimesters. Pregnancy may go unreported if the aviator leaves military service due to pregnancy. Despite these limitations, the initial survey found that 136 U.S. Army aviators were pregnant between January 1988 and December 1993, including 25 who were pregnant during 1993 (Shannon and Mason, 1994).

Open literature

Not much is known about the effects of WBV during pregnancy. One study reported a significant increase in stillbirth risk associated with maternal exposure to WBV (McDonald et al., 1988). Another study noted changes in the pelvic organs and lumbar spine among women exposed occupationally to low frequency WBV. The authors concluded, ". . . methodological problems with the measurement of dosage and with different biological effects in the female need to be overcome before making valid recommendations (World Health Organization, 1982)."

There is evidence that prenatal exposure to vibration adversely affects animal embryos. Exposure parameters, such as frequency, amplitude, duration of exposure, and time of exposure during pregnancy, may be critical to the outcome.

Two mammalian studies used the mouse model. Pregnant mice were exposed to vibration frequencies of 5, 10, and 20 Hz, 4.5 days after conception in the first study (Bantle, 1971). This timing was selected theorizing that the embryo would be most vulnerable during the implantation stage because of "the delicate balance of the synergistic action of estrogen and progesterone on the endometrium of the uterus for proper attachment of the embryo." The most damaging frequency was 20 Hz, the visceral resonance frequency of the mouse. At 20 Hz, a single exposure of 5.6 G_x for 10 minutes produced 10.2 percent malformations in the exposure group compared to only 1.6 percent in the control group (relative risk = 6.375). The surviving mouse pups in the exposure group had significantly lower birth weights than the control group pups.

In a second study, pregnant mice exposed to vibration of 3.5 G_z(rms) at 45 Hz for 4 hours had a 22.2 percent embryonic resorption rate. When the duration of exposure was increased to 8 hours, the resorption rate increased to 40.9 percent. The resorption rate was 5.6 percent for controls. Pathologic hematomas were present in the uteri of 5.9 percent of those mice receiving the 4 hours of exposure, and 22 percent of those mice receiving 8 hours of exposure, as compared to only 4

percent of the mice in the control group. The authors theorized that stress in the female animals produced a constriction of the pelvic blood vessels, impairing placental function and resulting in adverse pregnancy outcomes (Von Briese, Fanghanel, and Gasow, 1984).

Avian models were used to assess mortality risk associated with WBV during incubation. Eggs vibrated at 5 Hz with an amplitude of 0.25 G_x showed decreased embryonic oxygen uptake on 5th to 8th day of incubation (allantois development stage). The allantois expands from a small area of dense network of blood vessels on the 5th day to organ systems and covers the entire inner shell by the 9th day. Allantois functions include oxygen uptake and carbon dioxide elimination, replacing the vitelline circulation after the 5th day (Lizurek, 1973).

A study of Japanese quail eggs exposed before incubation to vibration frequencies of 5, 10, 20, 30, 50, 80, and 100 Hz showed increased mortality. The most significant effect occurred at 30 Hz where the mortality was 48.5 percent as compared to only 10.9 percent mortality in the control group. Considering mortality within the first 7 days of incubation, 10, 20, and 30 Hz had mortality rates of 7.82, 10.73, and 10.22 percent, respectively, versus 1.59 percent for the control group. In a second trial, mortality rates at 20 and 30 Hz were 6.39 and 9.17 percent, respectively, versus 3.9 percent for the control group. In both trials, vibration exposures of 50, 80, or 100 Hz, within the first 7 days of incubation, caused no more than 3.9 percent mortality among the embryos (Sabo, Boda, and Peter, 1982).

Chicken eggs were exposed to vibration frequencies of 1, 5, and 10 Hz for 15 minutes every 3 hours during incubation. The amplitude of exposure was 0.25 G_z for the 1-Hz group and 3.00 G_z for the 5- and 10-Hz groups. The resonant frequency of the egg yolk was 1.0 Hz, with no yolk motion observed below 0.6 Hz or above 1.7 Hz. Hatch rates were 84 percent in the control group, 54 percent in the 1-Hz group, 0 percent in the 5-Hz group, and 12 percent in the 10-Hz group. The authors concluded that vibration was lethal to developing chick embryos independent of the resonance motion of the yolk (Taggart, Alem, and Frear, 1990).

Model hypothesis

This project is centered on two concerns. Is there an increased risk for miscarriage and/or birth defects caused by exposure to WBV among pregnant women flying Army aircraft? There may be an association between the dependent variables (fetal death and/or congenital defect) and the independent variables (vibration frequency, amplitude, and exposure duration and timing). Our model construction was divided into four phases (Table 1). According to this model, pregnancy outcomes are highly dependent on exposure and the physiological response to the exposure. This study assessed and analyzed variation of vibration frequency and amplitude with fixed time of exposure in a chick embryo model.

Animal models are used for studying naturally-occurring human events. In our study, we suspect that the mortality and morbidity rates of our animal model will be affected by exposure dosage (vibration frequency and/or amplitude). Because of their short gestation, we used an avian

model in our HRA. Our hypothesis was: incubating chicken eggs exposed to WBV will have lower hatch rates than those not exposed to WBV.

Table 1.
Model construction.

| Phases | Description |
|--------|--|
| I. | Stage of development (timing of exposure during development) |
| II. | Amplitude, frequency, duration of exposure |
| III. | Physiologic response |
| IV. | Outcome |

We timed our WBV exposure to simulate maternal flying duties during the first and early second trimesters of pregnancy. A chicken lays its eggs about 24 to 27 hours after fertilization, when the blastoderm has differentiated into two layers by gastrulation. In a human, the bilaminar germ disc forms by 7.5 days after fertilization and by the 9th day the embryo is partially imbedded in the endometrial stroma. The chicken's heart begins to beat by the 42nd hour of incubation versus the 23rd day of human embryonic development. Limb formation starts at 62 to 64 hours of incubation in the chicken and by the 5th week in human. Feather germs appear during the 8th day of the chicken incubation while hair buds appear by the 4th month of human pregnancy. A chicken's beak forms from the 6th to 10th day of incubation, with the appearance of the beak evident by the 9th day. In human development, the fetus develops a human-looking face during the 3rd month (Lippincott, 1946; Langman, 1975; Stromberg, 1975). In our design, the timing of the first vibration exposure at 20 to 24 hours after the beginning of incubation should maximize the likelihood of teratologic effects to the developing internal organ systems, skull, and extremities.

Materials

A hydraulic single-axis Materials Test System (MTS[®]) table provided vibration input (Figure A-1 and Appendix C). A steel fixture, 36 inches by 36 inches by 0.75 inches with 36 threaded 0.625 inch holes equally spaced across its top, was secured to the table. A 0.25 inch metal plate was bolted to the fixture with 0.625 inch bolts. A varnished white pine box, approximately 8.5 inches by 20.5 inches by 6.0 inches, was centered and mounted with sheet metal screws to the metal plate. The box had an upper and lower half, connected by four metal door hinges which used O-type ring pins for easy removal of the upper half. The top of the upper box was covered with 0.5 inch by 0.5 inch wire mesh. A layer of mattress foam, approximately 1 inch thick, was placed immediately inside the wire mesh to hold the eggs in place. The bottom edge of the lower box had a 0.5 inch furring strip that was 1 inch from the bottom. The furring strip maintained the tray's position when the incubation tray was inserted into the lower half of the box (Figure A-2).

Two Humidaire™ model 21 self-turning incubators were used for chicken egg incubation. Lab support personnel made four smaller incubation trays to replace the original incubator trays (Figure A-3). Each incubator held four trays, 25 eggs per tray, for a total of 100 eggs (Figure A-4). The trays, measuring 6.5 inches by 18.5 inches by 3.5 inches, were constructed of varnished white pine. The bottom of each tray was made of 0.5 inch by 0.5 inch wire meshes and lined with egg crate mattress material (ECMM). The eggs were encased completely by ECMM for stability during vibration while an incubation tray was in the MTS® table fixture with the hinge pins secured. The ECMM is a commercial product placed between a mattress and bed linen. ECMM served two purposes during our study: (1) maintained the egg in an upright and stable position during incubation despite any manipulation, and (2) reduced the heat loss of the eggs while out of the incubator (Figure A-3). A 250-watt electric heat lamp was placed vertically 24 inches above the vibration table to further reduce heat loss. The tray design allowed easy removal and replacement of the trays from the incubator and provided the necessary stability for the eggs during handling.

Method

Experimental design

General principle

Vibration exposure may increase the likelihood of embryonic mortality. Incubating chicken eggs were exposed to vibration in a laboratory setting to evaluate the embryonic mortality risk. The initial study design used laboratory techniques previously employed in vibration studies. After the first run for each vibration frequency, the vibration amplitude dosage was increased if the previous dosage did not result in a significant mortality compared to controls or decreased if the prior dosage resulted in statistically significant mortality. The comparison was dose-response.

Egg care

Before each run, the incubators were disassembled and cleaned, following the manufacturer's instructions. The incubation trays and metal water container were autoclaved. The incubators were sprayed with a bacteriostatic solution and allowed to dry.

Conagra Poultry Company (CPC) furnished approximately 216 fertile eggs for each run. All eggs were candled upon arrival. Eggs with gross abnormalities in size or shape, cracks in the shell, or unusual air pockets or shadows were discarded. Less than 30 of about 1500 eggs were eliminated because of abnormalities detected during candling, mostly small cracks in the shell. After candling, 200 eggs were selected randomly for incubation. Before an incubation tray was filled with eggs, the bottom was lined with clean ECMM. Each egg was positioned within the tray and numbered by pencil to record its relative position within the tray. After the final egg was positioned, additional 1-inch spacers of ECMM were placed around each egg. The eggs were maintained for 12 to 24 hours at room temperature before beginning incubation.

For at least 24 hours before beginning incubation, the temperature and humidity within each incubator were stabilized at 99.5°F (Fahrenheit) and 84°F WB (wet bulb), respectively. The temperature and humidity were monitored every 12 hours throughout the run to ensure proper chick development. Humidity was maintained with distilled water. These conditions were maintained in the incubator until the last 4 days when the humidity was increased to between 90°F and 94°F WB. The increased humidity during the late stage of chick development prevents the growing chick from adhering to the membrane lining of the shell. Distilled water was applied to the eggs as required to support the hatching process.

The temperature of the laboratory room was increased to approximately 80°F to provide a more stable thermal environment for the eggs while they were on the MTS® table. The humidity at the MTS® table fixture was 80°F WB. No attempt was made to increase the room's humidity. A 250-watt heat lamp was positioned approximately 2 feet above the table to prevent cooling of the eggs during vibration. The egg yolk temperature in a trial egg was measured during 15 minutes (the protocol table exposure time) in the enclosed table fixture. The egg yolk temperature was maintained in the MTS® table setup.

Baseline run

The first of seven runs established a baseline mortality rate without vibration exposure. The protocol was followed, but the MTS® table was not vibrated.

During this run, eggs in one tray were broken when the incubator cycled while another tray was on the MTS® table. The Humidaire™ incubator has an electric motor connected to a timer. Twice daily, the motor turns incubating eggs by tilting the shelf holding the incubation trays. With one tray on the MTS® table, the tray remaining in the incubator shelf slid several inches and struck the side of the incubator with enough force to break several eggs. For the remaining runs, the electrical current was turned off to the external motor whenever a tray was removed from the incubator. After the trays were replaced, the motor was energized and the incubator cycled normally.

Experimental runs

The six remaining runs were experimental. Each of the two incubators held three experimental trays and one control tray with 25 eggs per tray. The control trays were handled the same as experimental trays except they were not exposed to vibration.

Eight exposure vibration frequencies were used: 5, 10, 15, 20, 25, 30, 40, and 50 Hz. The lowest frequency, 5 Hz, was selected based on a study that showed 5 Hz exposures inhibited embryo oxygen uptake (Lizurek, 1973). Higher frequencies were selected at 5 Hz increments up to 30 Hz, and then at 10 Hz increments up to 50 Hz. Vibration forces were applied in the G_z axis (up and down). Vibration amplitudes varied from 0.15 to 2.88 G_z (rms). The MTS® table was calibrated and tested before each run. The G_z amplitude was monitored during each run at one egg station.

Vibration frequencies and amplitudes were assigned to experimental trays before incubation. The outcome for each egg station in each tray was recorded. In subsequent runs, vibration amplitudes for a given vibration frequency were selected based on the experimental tray mortality rate observed in the prior run. If the mortality in an experimental tray was significantly greater than the control tray, the vibration amplitude was decreased for the next experimental tray exposed to the given vibration frequency. If the mortality in an experimental tray was not significantly greater than the control tray, the vibration amplitude was increased for the next experimental tray exposed to the given vibration frequency.

Vibration exposure began 20 to 24 hours after the start of incubation, modeling the 14th day postconception for the human embryo. Each exposure was administered by placing the incubation tray inside the aluminum fixture bolted to the MTS® vibration table. The exposure duration was 15 minutes at the predetermined vibration frequency and amplitude. Exposures were repeated every 4 hours through the 17th day of incubation. The duration and timing of the exposure, 15 minutes every 4 hours, were selected to model a pregnant woman flying a 2-hour mission, five times a week through the second trimester of pregnancy.

Each egg was candled during the second week of incubation to identify developing embryos during five of the six runs. All "clear" eggs and eggs with "blood lines" were identified. After incubation, all nonviable eggs were opened. Their contents were examined to identify the chick's stage of development and gross congenital abnormalities. Live chicks were weighed, examined for gross congenital abnormalities, and observed for activity level and muscle coordination.

Post-experiment validation runs

While the MTS® table had a metering device to measure the vibration amplitude generated by the table, this device did not measure the vibration amplitude within the egg. Eggs were encased in square wooden trays, surrounded by energy-attenuating ECMM. The trays were locked in a metal fixture on the MTS® table. This environment likely would alter the vibration exposure transmitted from the table to the egg. During the experimental runs, the egg vibration exposure was measured by affixing a piezoresistive accelerometer to one egg with collodion and bee's wax. The instrumented egg was placed within a tray of noninstrumented eggs (Figure A-3). The relative position of the instrumented egg within the tray was constant over all runs. Therefore, the exact exposure at each egg station within each tray was unknown.

We developed a post-experiment validation run to measure vibration amplitude at each egg station in a tray. Three dozen eggs of a similar size as the experimental eggs were obtained from the Fort Rucker commissary. These eggs were candled. We selected only eggs with normal structure, size, and shape. A piezoresistive accelerometer was attached to one egg with collodion and bee's wax. The instrumented egg was placed in the #1 position within the incubation tray. The tray was filled with eggs and ECMM. After filling, the tray was placed in a standard, temperature- and humidity-stabilized incubator. A second piezoresistive accelerometer was attached to the aluminum fixture so that the MTS® table could be monitored concurrently. After about 12 hours,

the incubation tray was removed from the incubator and secured within the fixture. The accelerometer cables were routed through an opening in the fixture's cover, through a charge amplifier, and finally connected to a Fluke® 77 multimeter. The accelerometer cable was secured throughout its length to limit artificial input to the signal.

We first tested the effect of tray orientation. Twenty-five eggs were put in an incubation tray. The MTS® table's controls initially were set to produce the lowest frequency and amplitude used previously in the study. The table was energized and the output of each accelerometer was recorded at 1 and 15 minutes. After 15 minutes of vibration, the table was turned off. The incubation tray was returned to the incubator. After 30 minutes, the incubation tray was placed in the fixture with 180 degrees of rotation from the first exposure. The table was energized and the output of each accelerometer was recorded at 1 and 15 minutes. This procedure was repeated for each combination of frequency and amplitude used in the experimental runs.

Second, we tested the effect of position in the tray on a single instrumented egg. The MTS® table's controls initially were set to produce the lowest frequency and amplitude used previously in the study. Using a tray with a temperature/humidity stabilized instrumented egg, the position of the egg was moved sequentially from the first position to another until all 25 positions were recorded. The trial was repeated moving the egg from the highest numbered position to the lowest number position. The accelerometer output was recorded twice at each egg station. This procedure was repeated for each combination of frequency and amplitude used in the experimental runs.

Next, we tested the effect of manipulating the instrumented egg between several exposures. The instrumented egg remained in the same egg station. One vibration frequency and amplitude was used in all exposures. After each exposure, the tray was returned to the incubator to stabilize the temperature and humidity.

Finally, we tested the effect of varying exposures on a single instrumented egg with other eggs in the tray. The MTS® table's controls initially were set to produce the lowest frequency and amplitude used previously in the study. Accelerometer outputs were measured at one egg station as the tray was exposed to all combinations of vibration frequency and amplitude used in the experimental runs. When the maximum dose was reached, the eggs were stabilized in the incubator. The test was repeated, this time going from the highest frequency and amplitude to the lowest.

Statistical analysis

Within an incubator, the number of hatches in an experimental tray was compared to the control tray in a 2X2 table. The effect of vibration was evaluated by chi-square and odds ratios. However, the post-experiment validation run showed that each egg station within a tray experienced a different vibration transmission ratio. So the unit of analysis became an egg station within a tray rather than a tray of eggs. While the binomial variable remained mortality, forward stepwise logistic regression were derived by SAS® LOGIST using position-specific exposure as the independent

variable. The logistic model assumed that the exposure at each egg station noted during the post-experiment validation run was duplicated in all trays during all experimental runs (SAS Institute, 1992).

Results

Baseline run

Table 2 shows the results from the baseline run. Analysis of variance showed there was no significant difference ($F=1.0$, $p=0.3632$) between the hatch rates of the seven surviving trays, and thus, between incubators (one tray was lost and not replaced, as described in methods section).

The mean hatch rate for the 7 surviving trays was 84 percent. Conagra Commercial Hatchery reported a hatch rate of 88 percent for the same flock/day. A comparison of means showed no significant difference between the mean hatch rates of the baseline run eggs and the Conagra flock eggs (one-tailed test of significance, $p=0.08$).

Table 2.
Observed hatch rate, overall and tray-specific, during the validation phase.

| Tray | Eggs | Hatch | Nonhatch | % Mortality |
|-------|---|-------|----------|-------------|
| 1 | 25 | 21 | 4 | 16 |
| 2 | 25 | 23 | 2 | 8 |
| 3 | Removed from study (see method section) | | | |
| 4 | 25 | 21 | 4 | 16 |
| 5 | 25 | 20 | 5 | 20 |
| 6 | 25 | 19 | 6 | 24 |
| 7 | 25 | 20 | 5 | 20 |
| 8 | 25 | 23 | 2 | 8 |
| Total | 175 | 147 | 28 | 16 |

Experimental runs

We incubated 1200 eggs, using 200 per experimental run. Only nine eggs were cracked during the protocol. These eggs were dropped from the analysis since a cracked shell may increase the introduction of bacteria and increase the loss of humidity, both resulting in higher risk for mortality independent of vibration exposure. The analytical database contained 1,191 eggs.

During the first experimental run, one control tray was exposed accidentally to vibration (15 Hz, 3.0 G_z) for several minutes. The observed hatch rate for this tray was less than 50 percent while the rate for the other control tray was 92 percent. While this control tray was dropped from our analyses, the incident showed that a single vibration exposure increased the fetal mortality risk.

Table 3 shows the mortality outcome for egg incubation trays that were exposed to 30 combinations of vibration frequencies (Hz) and amplitudes (G_z(rms)). The overall mortality rate was 31.9 percent. The mortality by tray ranged from 12 percent at 25 Hz, 0.18 G_z(rms) to 100 percent at 15 Hz, 2.86 G_z(rms). As shown in Table 3, an amplitude of 0.90 G_z(rms) with a frequency of 25 Hz exposure resulted in 44 percent mortality. A 30-Hz exposure more than doubled the mortality to 92 percent mortality ($p < 0.05$). At 15 Hz, a 0.65 G_z(rms) exposure resulted in 16 percent mortality and a 1.5 G_z(rms) exposure approximately doubled the mortality to 31 percent (Table 3). When the amplitude was again nearly to 2.88 G_z(rms), the mortality tripled to 100 percent.

In the 25 Hz range, an exposure of 0.15 G_z(rms) resulted in 24 percent mortality (Table 3). Nearly doubling the vibration exposure to 0.26 G_z(rms), almost doubled the mortality to 44 percent. When the G_z(rms) was increased to 0.77 G_z(rms) and 0.90 G_z(rms), the mortality remained unchanged. When the exposure was increased to 1.27 G_z(rms), more than 7 times the 0.15 G_z(rms) exposure, the mortality increased to 96 percent.

Post-experiment validation runs

For a given position in a tray, none of the accelerometer outputs varied by more than 2 mV in all of the post-experiment validation procedures. This left the position of the egg within the tray as a potential confounder. Using the G_z(rms) fixture as the exposure variable yielded unsatisfactory results in regions of the data where table to egg transmission rates were high. The vibration transmission was computed as the ratio of the G_z(rms) egg to G_z(rms) table. When the vibration transmission was above 1.5, the ability of the G_z(rms) fixture to predict mortality was compromised.

For some exposure settings, the egg accelerometer output varied in the same tray by more than 500 percent from one egg position to another as shown in Table 4 and Table 5. In the most extreme case, only 20 percent of the table's energy was measured at one egg position while at another position within the same tray, energy was measured at 174 percent. Because of the absence of homogeneity within exposure, the original analysis plan was modified so that the egg position, rather than the tray, became our analytical unit.

Table 3.
Chick mortality versus vibration exposure in the experimental runs.

| Frequency Hz | Amplitude G_z (rms) table | Mortality rate per 100 eggs |
|--------------|-----------------------------|-----------------------------|
| 5 | 2.00 | 20 |
| | 2.33 | 36 |
| 10 | 1.90 | 24 |
| | 2.33 | 44* |
| 15 | 0.65 | 16 |
| | 1.50 | 31 |
| | 2.88 | 100* |
| 20 | 0.43 | 16 |
| | 0.98 | 40* |
| | 1.06 | 40* |
| | 2.09 | 92* |
| 25 | 0.15 | 24 |
| | 0.18 | 12 |
| | 0.26 | 44* |
| | 0.77 | 36* |
| | 0.90 | 44* |
| | 1.27 | 96* |
| 30 | 0.15 | 16 |
| | 0.47 | 52* |
| | 0.51 | 16 |
| | 0.52 | 64* |
| | 0.90 | 92* |
| 40 | 0.18 | 32 |
| | 0.63 | 52* |
| 45 | 0.29 | 24 |
| | 0.38 | 28 |
| | 0.53 | 44* |
| 50 | 0.28 | 20 |
| | 0.47 | 32 |
| | 0.95 | 72* |

* Significantly different than control group, $\alpha = 0.05$.

Table 4.
 Energy at the egg expressed in terms of the minimum, maximum,
 and mean $G_z(\text{rms})$ for each exposure level.

| Frequency Hz | Amplitude $G_z(\text{rms})$ table | Amplitude $G_z(\text{rms})$ egg | | |
|--------------|--------------------------------------|---------------------------------|---------|------|
| | | Minimum | Maximum | Mean |
| 5 | 2.00 | 1.31 | 1.57 | 1.37 |
| | 2.33 | 1.52 | 1.64 | 1.59 |
| 10 | 1.90 | 1.28 | 1.41 | 1.35 |
| | 2.33 | 1.57 | 1.73 | 1.65 |
| 15 | 0.65 | 0.47 | 0.65 | 0.55 |
| | 1.50 | 1.14 | 1.38 | 1.26 |
| | 2.86 | 2.27 | 2.74 | 2.51 |
| 20 | 0.43 | 0.31 | 0.40 | 0.36 |
| | 0.98 | 0.84 | 1.25 | 1.02 |
| | 1.05 | 0.98 | 1.38 | 1.10 |
| | 2.09 | 1.86 | 2.81 | 2.31 |
| 25 | 0.15 | 0.09 | 0.14 | 0.11 |
| | 0.18 | 0.16 | 0.27 | 0.22 |
| | 0.26 | 0.23 | 0.39 | 0.30 |
| | 0.77 | 0.75 | 1.33 | 1.02 |
| | 0.90 | 0.89 | 1.68 | 1.26 |
| | 1.27 | 1.47 | 3.01 | 2.19 |
| 30 | 0.15 | 0.22 | 0.46 | 0.33 |
| | 0.47 | 0.75 | 2.42 | 1.54 |
| | 0.51 | 0.68 | 2.42 | 1.50 |
| | 0.52 | 0.78 | 2.72 | 1.58 |
| | 0.90 | 1.54 | 4.93 | 2.70 |
| 40 | 0.18 | 0.18 | 0.81 | 0.39 |
| | 0.63 | 0.65 | 2.16 | 1.09 |
| 45 | 0.29 | 0.20 | 0.51 | 0.37 |
| | 0.38 | 0.25 | 0.72 | 0.48 |
| | 0.53 | 0.27 | 1.27 | 0.79 |
| 50 | 0.28 | 0.07 | 0.44 | 0.22 |
| | 0.29 | 0.10 | 0.83 | 0.42 |
| | 0.47 | 0.19 | 1.65 | 0.80 |

Table 5.

The distribution of the amplitude transmission ratios within the fixture-egg unit expressed as the minimum, maximum, and mean value.

| Frequency Hz | Amplitude G_z (rms) table | Amplitude transmission ratios (egg/table) | | |
|--------------|--------------------------------|---|---------|------|
| | | Minimum | Maximum | Mean |
| 5 | 2.00 | 0.65 | 0.78 | 0.68 |
| | 2.33 | 0.65 | 0.71 | 0.61 |
| 10 | 1.90 | 0.67 | 0.74 | 0.71 |
| | 2.33 | 0.68 | 0.74 | 0.71 |
| 15 | 0.65 | 0.72 | 1.00 | 0.84 |
| | 1.50 | 0.76 | 0.93 | 0.84 |
| | 2.86 | 0.79 | 0.95 | 0.87 |
| 20 | 0.43 | 0.72 | 0.93 | 0.83 |
| | 0.98 | 0.86 | 1.28 | 1.04 |
| | 1.05 | 0.92 | 1.30 | 1.08 |
| | 2.09 | 0.89 | 1.35 | 1.11 |
| 25 | 0.15 | 0.59 | 0.88 | 0.72 |
| | 0.18 | 0.86 | 1.49 | 1.18 |
| | 0.26 | 0.88 | 1.50 | 1.16 |
| | 0.77 | 0.97 | 1.73 | 1.32 |
| | 0.90 | 0.99 | 1.86 | 1.40 |
| | 1.27 | 1.16 | 2.37 | 1.73 |
| 30 | 0.15 | 1.41 | 2.97 | 2.17 |
| | 0.47 | 1.60 | 5.14 | 3.27 |
| | 0.51 | 1.34 | 4.75 | 2.95 |
| | 0.52 | 1.50 | 5.23 | 3.03 |
| | 0.90 | 1.70 | 5.46 | 2.99 |
| 40 | 0.18 | 0.99 | 4.43 | 2.16 |
| | 0.63 | 1.04 | 3.45 | 1.75 |
| 45 | 0.29 | 0.71 | 1.75 | 1.29 |
| | 0.38 | 0.66 | 1.88 | 1.25 |
| | 0.53 | 0.51 | 2.40 | 1.50 |
| 50 | 0.28 | 0.24 | 1.57 | 0.80 |
| | 0.29 | 0.22 | 1.77 | 0.90 |
| | 0.47 | 0.20 | 1.74 | 0.84 |

In our regression model, the dependent variable was hatch (No=1, Yes=0). The independent variables were G_z (rms) fixture, G_z (rms) egg, table frequency (Hz), and the computed value for the table to egg amplitude transmission ratio at each egg station.

By definition, a threshold is evident when an effect is noted above a point but not below the point. In regression models, a threshold changes the slope of the regression line at the threshold value. During logistic regression modeling, no threshold value was found which significantly affected the fit of the model for any of the covariates.

The final logistic regression model was:

| Variable | Estimate | SE | Wald χ^2 | p |
|--------------------|----------|--------|---------------|--------|
| Intercept | -1.7764 | 0.1289 | 189.84 | 0.0001 |
| G_z (RMS) eggs | +2.6846 | 0.3649 | 54.13 | 0.0001 |
| G_z (RMS) tables | -1.0657 | 0.2666 | 15.97 | 0.0001 |
| Hz | +0.0331 | 0.0055 | 36.80 | 0.0001 |
| Resonance | -0.9621 | 0.1936 | 24.69 | 0.0001 |

The fit of the model was: concordance, 70.4 percent; discordance, 21.1 percent; and 8.6 percent were tied.

To determine the mortality effect of a vibration amplitude for a given frequency and transmission ratio, consider the following two calculations as examples. At an exposure vibration frequency of 5 Hz, an amplitude transmission ratio of 1.0, and amplitude of 1.0 G_z (rms), the logistic model predicts a 27.8 percent mortality. The calculation was:

$$\text{Log odds} = -1.77647 + (1.0(2.6846)) + (1.0(-1.066)) + (5.0(0.0331)) + (1.0(-0.9621)) = -0.9544.$$

$$\text{Odds} = e^{-0.9544} = 0.385.$$

$$\text{Probability of death for any chick} = \frac{\text{odds}}{1+\text{odds}} = 0.278.$$

For a frequency of 5 Hz and an amplitude transmission ratio at 1.0, but increasing the amplitude to 1.5 G_z (rms) at the egg, the logistic model predicts a mortality of 46.4 percent. The calculation was:

$$\text{Log odds} = -1.77647 + (1.5(2.6846)) + (1.5(-1.066)) + (5.0(0.0331)) + (1.0(-0.9621)) = -0.1452.$$

$$\text{Odds} = e^{-0.1452} = 0.865.$$

$$\text{Probability of death for any chick} = \frac{\text{odds}}{1+\text{odds}} = 0.464.$$

At a single egg station exposed to a vibration frequency of 5 Hz, modifying the vibration amplitude from 1.0 to 1.5 G_z (rms), increases the predicted mortality rate from 27.8 percent to 46.4 percent, almost doubling the predicted mortality. If the exposure is changed to an amplitude of 3.0 G_z (rms), the predicted mortality rate increases to 90.7 percent. Doubling the vibration amplitude doubles the predicted mortality rate at 5 Hz.

Modifying the vibration frequency from 5 Hz to 30 Hz at a single egg station exposed to a vibration amplitude of 1.5 G_z (rms) with an amplitude transmission ratio of 1.0, increased the predicted mortality from 46.4 percent to 66.4 percent.

Using the logistic model, we can consider the effect of amplitude transmission on mortality at various egg stations. For example, at an exposure frequency of 5 Hz and amplitude of 1.5 G_z (rms), the predicted mortality rates would be 15.7 percent for an amplitude transmission ratio of 0.5, 46 percent for a transmission ratio of 1.0, and 94 percent for a ratio of 2.0. Thus, different egg stations at a given vibration frequency and table amplitude, experienced varying mortalities if the transmission ratios were different at these egg stations.

Figure 1 shows the relationship between predicted mortality and exposure vibration frequency and amplitude controlling for amplitude transmission.

Chick examination findings

Among the hatching chicks, we observed no significant effects of vibration on hatch weight. We observed 6 chicks with congenital defects in the exposure group of 900 eggs versus none in the control group of 300 eggs ($p < .001$). All of the chicks with congenital defects had vibration exposures greater than 20 Hz. The most common congenital syndrome was a crossed beak, undeveloped eye, and missing bony structures in the skull found in four chicks. None with these severe malformations occurred at frequencies less than 20 Hz nor at acceleration amplitudes less than 3.0 G_z (rms) as measured at the egg. Since the beak begins to form on the 6th day of incubation and is completely formed by the 10th day of incubation, these findings were consistent with a possible teratogenic effect for vibration exposure at high (20 Hz or larger) frequencies. High mortality rates, often exceeding 90 percent, were associated with high exposure and compromised our ability to study the teratogenic effect of vibration.

In addition, we observed experimental chicks with malformed feet, sensory disorientation, and muscular weakness. They were unable to walk, or even stand, hours after hatching. These conditions were only observed among the experimental trays, not among the hundreds of control chicks. Disorientation and muscular weakness were noted in the 45 Hz and 50 Hz groups independently by all three observers.

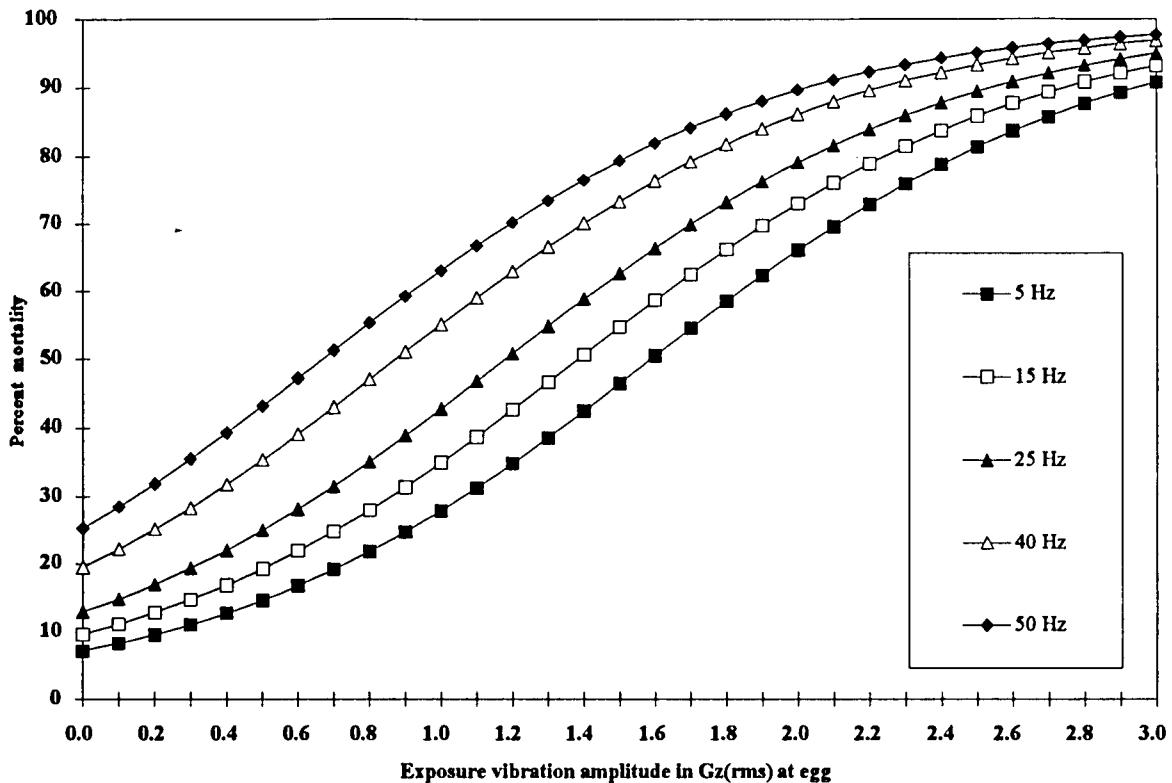


Figure 1. Mortality versus exposure frequency (Hz) and amplitude, controlling for differences in amplitude transmission ratios between egg stations.

Discussion

Our focus on modeling expanded the knowledge of vibration exposure and its effect on the developing chicken embryo. We believe modeling is useful even when the data are limited. We cannot study directly human development in vibration environments, since there are known and unknown difficulties in obtaining such measurements. To date, human exposure data has been limited to a few environmental measurements, such as accelerometers attached to an aviator's skin, head gear, and seat system. Given the complex mix of vibrations in the aviation environment, it would be difficult to identify specific vibration exposure that would harm the developing human. Knowing animal models are affected adversely by vibration increases our concern that exposure to vibration may result in adverse effects for pregnant women.

All health risk assessment studies have inherent strengths and limitations. A major limitation of this study is that the mount system was assessed retrospectively, rather than prospectively. Differences in amplitude transmission at each egg station complicated our analyses. We did not study transmission in the mount system to determine its origin. We studied amplitude transmission in post-experiment validation runs to explain the differences in the risk estimates we observed using entire egg trays for the measure of mortality risk.

There was good agreement between accelerometer measurements showing the amplitude transmission was stable over time for a specific egg position. The transmission was not affected by the mass of the instrumented egg or the mass of the eggs surrounding the instrumented egg. We do acknowledge that another mount system was required, which minimized the differences in amplitude transmission between egg stations within the tray/table fixture unit. In future studies, we recommend prospectively validating other egg containers, such as a circular mount fixture and a circular incubation tray. We also recommend prospectively validating the transmission rates between each tray to rule out differences caused by tray construction and ECMM.

Other mortality risks were not included in the logistic model because their effects are known or believed to operate through other covariates that are in the model. For example, fetal mortality is correlated with advanced maternal age. The effects of flock's age on chick mortality largely is mediated by CPC's policy of limiting the age of the hens. Similarly, abnormal hatch rates can be affected by periods of extremes in environmental temperature in CPC's hatchery.

Conclusions

Factors associated with chicken embryo mortality were: frequency, amplitude, amplitude transmission, and timing of the exposure. As the magnitude of the exposure increased, mortality increased. No clear HRA threshold values were identified since we discovered protocol problems retrospectively. The use of an entire tray of eggs as the unit of amplitude measure was invalid due to the differences in vibration amplitude transmission at each egg station. However, mortality thresholds were proposed using a logistic model that controlled for the differences in vibration amplitude noted at various egg stations. No extraneous factors such as month of incubation, flock, and incubator were significant in analysis of variance modeling.

Controlling for the differences in amplitude transmission between egg stations, we found very strong effects of vibration on embryonic development. Chicken embryos should not be exposed to vibration above 2.0 G_z . Exposures as low as 1.0 G_z are harmful at certain frequencies.

Congenital malformations occurred in chicks exposed to vibration, but in none of the control chicks. These malformations were a syndrome of crossed beaks and missing eyes. We observed chicks with malformed feet, sensory disorientation, and muscular weakness. Further studies are required to define and validate these findings.

Whole-body vibration exposures are harmful to the developing chicken embryo. Until further laboratory and epidemiological studies of this potential health hazard on animal and human pregnancy outcomes are completed, pregnant Army aviators should not fly in rotary-wing aircraft. We believe the vibratory aspect of the rotary-wing aircraft environment is a fetal health hazard. Our current collective opinion supports the Army policy of restricting pregnant aviators from rotary-wing flying duties.

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Appendix A
Figures of study material

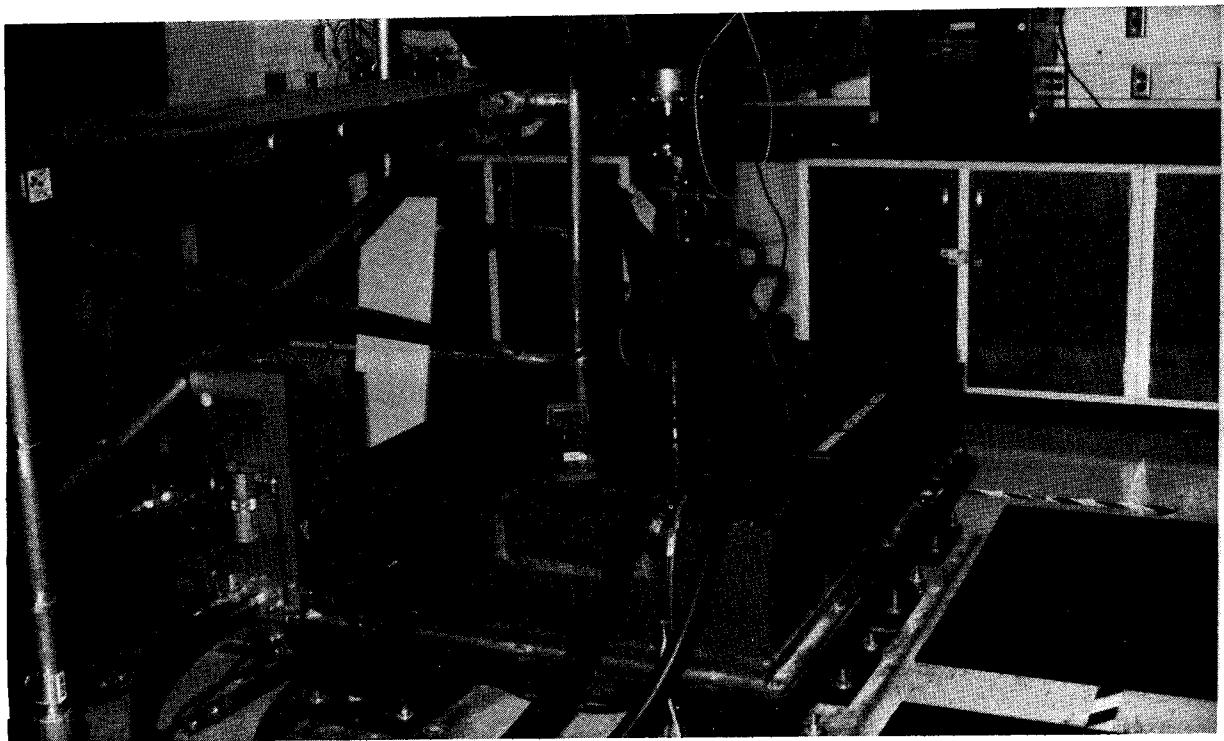


Figure A-1. MTS® table used in the study.

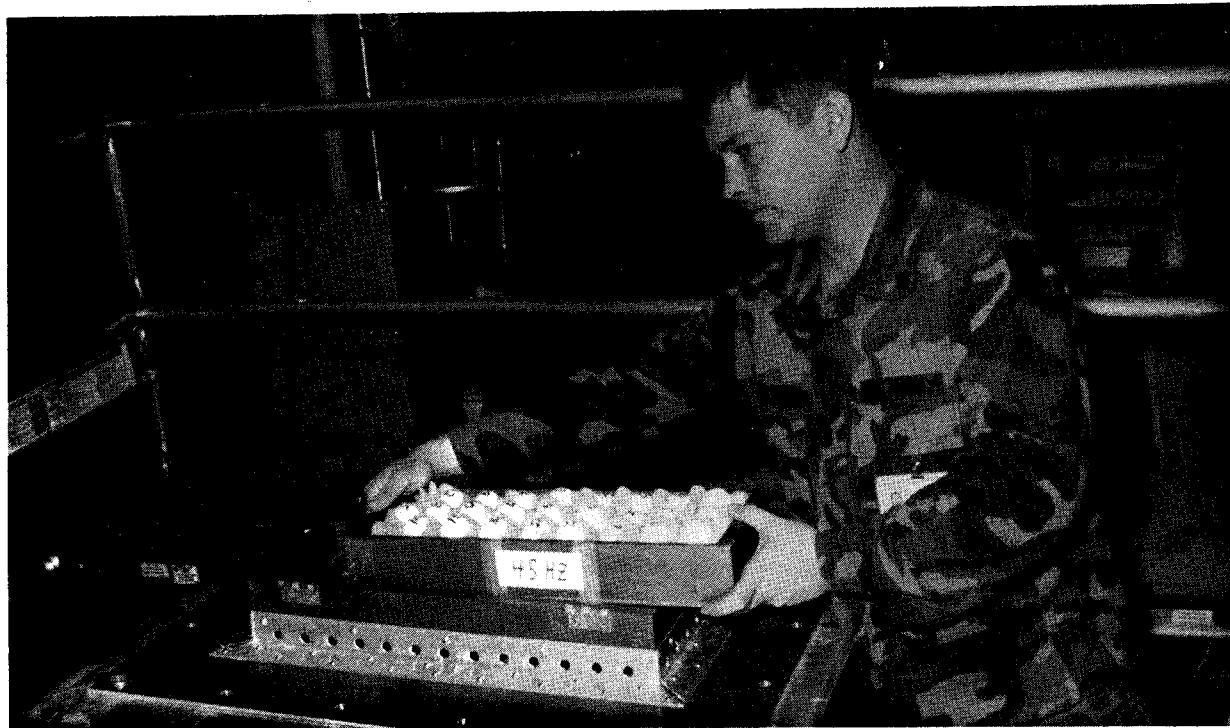


Figure A-2. Top of MTS® table showing metal fixture and the bottom of wooden tray used to incubate eggs. For illustration purposes only, the eggs were numbered with a felt-tipped black marker.

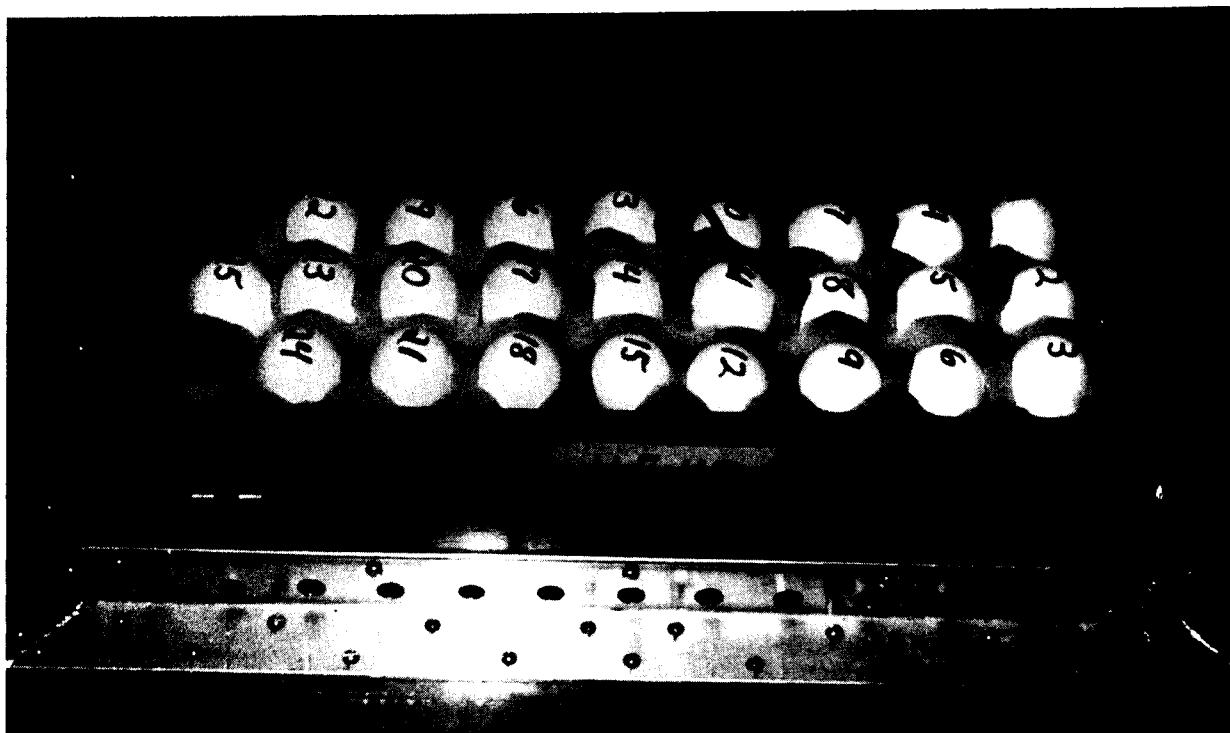


Figure A-3. Wooden incubation tray showing piezoresistive accelerometer attached to egg #11.



Figure A-4. Humidaire™ model 21 incubator, showing position of wooden incubation trays.

Appendix B.
Abbreviations used

| Abbreviation | meaning |
|----------------|-------------------------------|
| 1. χ^2 | chi-square |
| 2. CPC | Conagra Poultry Company |
| 3. e | exponential |
| 4. ECMM | egg craft mattress material |
| 5. F | Fahrenheit |
| 6. G_z (rms) | amplitude in G_z axis (rms) |
| 7. HRA | health risk assessment |
| 8. Hz | hertz |
| 9. mm | millimeter |
| 10. mV | millivolt |
| 11. SE | standard error |
| 12. s.d. | standard deviation |
| 13. WB | wet bulb |
| 14. WBV | whole-body vibration |
| 15. WHO | World Health Organization |

Appendix C.
Equipment manufacturers' list

Bruel & Kjaer Instruments, Inc.
185 Forest Street
Marlboro, MA 01752

Genrad
300 Baker Avenue
Concord, MA 02254

Hewlett-Packard Company
4700 Bayou Boulevard
Pensacola, FL 32502

Humidaire Incubator Company
217 West Wayne Street
New Madison, OH 45346

Kistler Instrument Corporation
75 John Glen Drive
Amherst, NY 14120-5019

Larsen-Davis Laboratories
280 South Main
Pleasant Grove, UT 84062

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Fort Monmouth, NJ 07703-5305

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FAA Technical Center
Atlantic City, NJ 08405

Director
Walter Reed Army Institute of Research
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Commander, U.S. Army Test and Evaluation Command
Directorate for Test and Evaluation
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Aberdeen Proving Ground,
MD 21010-5425

Commander
USAMRMC
ATTN: SGRD-RMS
Fort Detrick, Frederick, MD 21702-5012

HQ DA (DASG-PSP-O)
5109 Leesburg Pike
Falls Church, VA 22041-3258

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Office of Naval Research
600 North Quincy Street
Arlington, VA 22217

Commandant
U.S. Army Aviation Logistics School
ATTN: ATSQ-TDN
Fort Eustis, VA 23604

Headquarters (ATMD)
U.S. Army Training and Doctrine Command
ATTN: ATBO-M
Fort Monroe, VA 23651

IAF Liaison Officer for Safety
USAF Safety Agency/SEFF
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Kirtland Air Force Base
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HQ USCENTCOM (CCSG)
U.S. Central Command
MacDill Air Force Base, FL 33608

Director
Directorate of Combat Developments
ATTN: ATZQ-CD
Building 515
Fort Rucker, AL 36362

U.S. Air Force Institute
of Technology (AFIT/LDEE)
Building 640, Area B
Wright-Patterson
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Director, Institute of Aviation
University of Illinois-Willard Airport
Savoy, IL 61874

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Arlington Hall Station
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Arlington, VA 22204-1382

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ATTN: CEWES-IM-MI-R,
CD Department
3909 Halls Ferry Road
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